

BIONETICS

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MUTAGENICITY EVALUATION

OF

FDA 75-79

VITAMIN A

ACETATE CRYSTALS

FINAL REPORT

VITAMIN A / ACETATE
CRYSTALS
Final Report
Sept. 1997

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5516 Nicholson Lane
Kensington, Maryland
20795

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FDA 75-79

VITAMIN A

ACETATE CRYSTALS

FINAL REPORT

SUBMITTED TO

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DIVISION OF TOXICOLOGY
BUREAU OF FOODS
U.S. FOOD AND DRUG ADMINISTRATION
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LBI PROJECT NO. 2672

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EVALUATION SUMMARY

The test compound, FDA 75-79, Vitamin A acetate crystals, did not exhibit mutagenic activity in any of the assays employed in these studies.



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DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-79, Vitamin A acetate crystals

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: October 29, 1976
2. Description: Pale yellow flaky powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 μ moles
2. Glucose-6-phosphate	5 μ moles
3. Sodium phosphate (dibasic)	100 μ moles
4. $MgCl_2$	8 μ moles
5. KCl	33 μ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	



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D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical^a</u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS ^b
	Ethylmethanesulfonate	Water or saline	BPS ^b
	2-Nitrofluorene	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard	Water or saline	FS ^b
Activation	Dimethylnitrosamine	Water or saline	BPS ^b
	2-Acetylaminofluorene	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline	Dimethylsulfoxide ^c	FS ^b
	2-Aminoanthracene	Dimethylsulfoxide ^c	BPS ^b

^a Concentrations given in the Results Section

^b BPS = base-pair substitution; FS = frameshift

^c Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



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B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.



IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-79, Vitamin A acetate crystals
2. Test solvent: *DMSO
3. Solubility of the test compound under treatment conditions: Soluble
4. Additional comments: Pale yellow flaky powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: December 22, 1976
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.0625	0.425
1/2 50% Survival	0.1250	0.850
50% Survival	0.2500	1.700

*The concentration of solvent was equal to the highest volume of test material added.



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C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000127479
 B. TEST DATE: APRIL 8, 1977

TEST	SPECIES	ISSUE	BEVERIANIS PER PLATE									
			TA-1535		TA-1537		TA-1538		TA-98		TA-100	
			1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION												
SOLVENT CONTROL*	---	---	11	18	11	16	10	19	37	51	270	263
POSITIVE CONTROL**	---	---	>1000	>1000	>1000	>1000	773	823	>1000	>1000	800	728
TEST 0.25000 %	---	---	19	10	20	12	11	12	32	26	208	202
0.12500 %	---	---	10	13	18	17	10	10	24	53	130	101
0.06250 %	---	---	15	12	10	16	11	13	39	56	63	121
2. ACTIVATION												
SOLVENT CONTROL*	MOUSE	LIVER	25	22	18	10	30	22	35	39	129	148
	RAT	LIVER	34	27	21	19	18	20	37	40	128	155
	MONKEY	LIVER	25	26	13	17	18	17	42	39	117	148
POSITIVE CONTROL***	MOUSE	LIVER	848	>1000	207	766	>1000	>1000	>1000	>1000	797	871
	RAT	LIVER	353	388	621	434	>1000	>1000	>1000	>1000	>1000	>1000
	MONKEY	LIVER	204	319	511	215	665	871	>1000	>1000	>1000	>1000
TEST 0.25000 %	MOUSE	LIVER	11	12	12	23	21	24	38	42	81	70
0.12500 %	MOUSE	LIVER	16	19	17	20	19	16	32	44	131	133
0.06250 %	MOUSE	LIVER	13	12	10	13	24	22	34	38	113	137
0.25000 %	RAT	LIVER	15	16	19	20	32	25	32	41	115	101
0.12500 %	RAT	LIVER	16	13	11	11	14	30	41	30	140	134
0.06250 %	RAT	LIVER	13	15	12	11	19	16	29	53	125	114
0.25000 %	MONKEY	LIVER	12	18	10	11	20	17	46	38	146	81
0.12500 %	MONKEY	LIVER	19	18	16	11	19	15	49	53	128	138
0.06250 %	MONKEY	LIVER	19	14	11	10	25	24	50	41	128	127

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
 TA-1537 QM 20 UG/PLATE
 TA-1538 NF 100 UG/PLATE
 TA-98 NF 100 UG/PLATE
 TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
 TA-1537 AMQ 100 UG/PLATE
 TA-1538 AAF 100 UG/PLATE
 TA-98 AAF 100 UG/PLATE
 TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

NONACTIVATION COMPOUND 000127479

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
NAN		101.71	5.40	8.45	6.64	27.61	18.25	11.73	CONTROLS
NAP		2775.23	1203.47	611.11	161.67	154.04	160.82	124.93	
<hr/>									
NA1		10.79	3.12	4.28	6.26	4.15	25.12	10.90	TEST DATA
NA2		12.02	3.54	3.36	6.62	6.73	20.65	7.61	
NA3		75.80	5.22	3.99	6.95	4.63	18.72	7.76	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

SPECIES ICRFLO/MOUSE COMPOUND 000127479

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	78.09	3.81	6.87	15.20	9.69	1.42	0.87	NEGATIVE CONTROLS
ACT	A-C	90.02	5.81	7.88	18.56	14.07	2.23	1.78	
ACT	ALI	92.08	5.02	7.30	12.42	30.17	8.45	3.69	
ACT	ALU	85.69	5.12	7.14	19.44	20.99	6.55	4.68	
<hr/>									
ACT	PLI	210.13	162.45	94.83	412.57	135.88	139.35	75.03	POSITIVE CONTROLS
ACT	PLU	87.12	7.85	5.79	87.74	56.13	8.25	2.71	
<hr/>									
ACT	L11	30.32	4.35	2.54	12.72	15.08	3.83	1.28	TEST COMPOUND
ACT	L12	99.34	3.11	2.86	17.62	13.71	4.64	0.88	
ACT	L13	84.56	4.24	3.43	10.05	11.57	4.90	0.98	
ACT	LU1	43.66	3.83	2.43	13.28	8.67	1.90	0.55	
ACT	LU2	62.09	2.35	1.87	10.05	2.67	3.48	1.03	
ACT	LU3	58.21	3.09	5.03	11.37	22.16	2.97	0.77	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

SPECIES SPRDAW/RAT COMPOUND 000127479

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	94.89	8.04	7.37	3.96	8.23	13.27	8.24	NEGATIVE CONTROLS
ACT	A-C	62.59	6.10	1.71	6.48	5.63	22.68	9.82	
ACT	ALI	92.83	10.14	3.54	6.79	40.57	27.77	15.41	
ACT	ALU	102.72	12.87	4.77	9.03	35.33	24.60	11.70	
<hr/>									
ACT	PLI	284.17	189.41	62.91	100.99	235.81	84.59	45.78	POSITIVE CONTROLS
ACT	PLU	92.32	9.42	2.79	110.98	173.22	16.03	11.02	
<hr/>									
ACT	LI1	69.67	8.70	4.11	6.61	9.72	13.33	6.06	TEST COMPOUND
ACT	LI2	85.16	8.40	2.48	9.58	18.03	13.92	7.64	
ACT	LI3	106.90	4.21	4.82	7.20	15.86	15.44	10.34	
ACT	LU1	61.90	6.33	2.67	8.65	9.87	12.41	6.28	
ACT	LU2	86.57	15.32	3.54	7.17	20.97	16.83	8.22	
ACT	LU3	86.75	6.58	7.05	5.31	12.18	14.67	6.67	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

SPECIES RHESUS/MONKEY COMPOUND 000127479

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	71.22	15.03	3.16	1.78	7.97	20.69	8.30	NEGATIVE CONTROLS
ACT	A-C	24.59	9.17	4.03	2.76	8.66	17.36	7.70	
ACT	ALI	80.81	13.76	5.68	5.01	31.01	15.76	9.19	
ACT	ALU	74.62	17.42	4.33	8.42	16.75	20.62	8.94	
<hr/>									
ACT	PLI	173.50	90.35	57.70	114.16	672.39	88.34	62.48	POSITIVE CONTROLS
ACT	PLU	73.62	12.79	2.79	5.01	8.68	16.17	11.98	
<hr/>									
ACT	LI1	6.75	8.26	3.22	10.00	12.50	13.98	6.55	TEST COMPOUND
ACT	LI2	50.44	6.11	4.07	11.80	18.15	24.65	6.98	
ACT	LI3	75.91	5.94	2.49	8.50	18.02	17.15	9.28	
ACT	LU1	2.53	9.60	3.41	8.31	7.12	14.09	6.34	
ACT	LU2	61.61	7.27	3.58	11.30	7.50	15.47	8.19	
ACT	LU3	68.81	8.19	4.04	8.47	6.04	14.24	6.45	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	<p> NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) </p> <p> A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test </p> <p> LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels </p>
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan



V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-79, Vitamin A acetate crystals, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

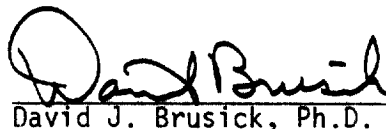
2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 75-79, Vitamin A acetate crystals, did not exhibit mutagenic activity in any of the assays employed in these studies.


Submitted by:


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7/29/77
Date

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Robert J. Weir, Ph.D.
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VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or revertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

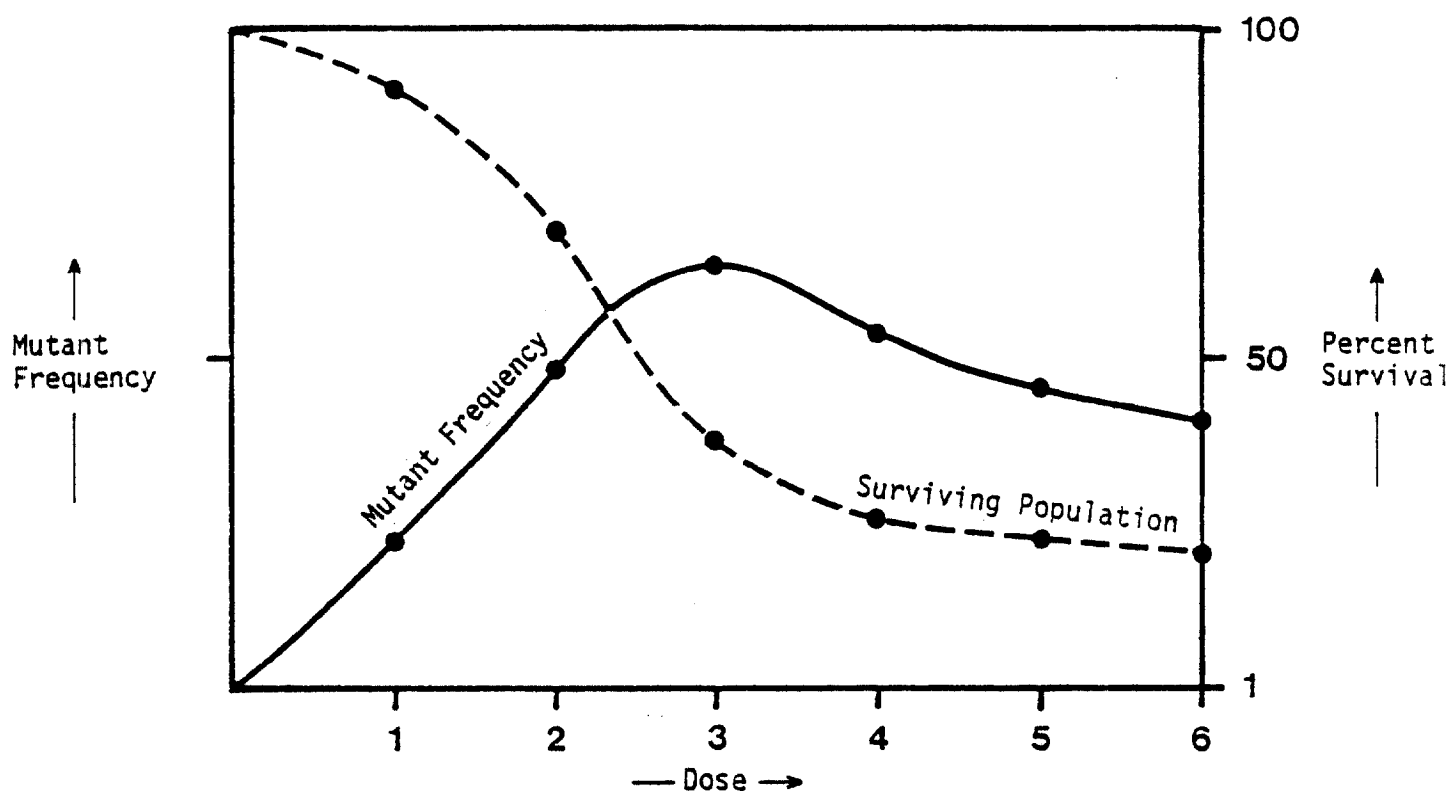
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

OBSERVED DOSE RESPONSE

A typical positive dose response set of data would be obtained.

The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.

Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX
Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/25/77			
EXPERIMENT 636504	DETECTOR TA100	SPECIES /					
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0351	0357	101.71	0
	NAP		EMS 0.066%	0218	6050	2775.23	0
000127479	NA1		0025-2 PCT.	0139	0015	10.79	0
000127479	NA2		0125-3 PCT.	0258	0031	12.02	0
000127479	NA3		0625-4 PCT.	0405	0307	75.80	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/25/77			
EXPERIMENT 708102	DETECTOR TA1535	SPECIES					
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0537	0029	5.40	0
	NAP		EMS 0.2%	0605	7281	1203.47	0
000127479	NA1		0025-2 PCT.	0449	0014	3.12	0
000127479	NA2		0125-3 PCT.	0452	0016	3.54	0
000127479	NA3		0625-4 PCT.	0594	0031	5.22	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/25/77			
EXPERIMENT 636201		DETECTOR TA1537		SPECIES /			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SOLVENT	0213	0018	8.45	0
		NAP	QM 13 UG/ML	0063	0385	611.11	0
000127479	NA1		0025-2 PCT.	0397	0017	4.28	0
000127479	NA2		0125-3 PCT.	0476	0016	3.36	0
000127479	NA3		0625-4 PCT.	0451	0018	3.99	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102			PROJECT 2672		DATE - 07/25/77		
EXPERIMENT 705401		DETECTOR TA1538	SPECIES /				
COMPOUND	TEST	ORG ID	CONCENTRATION	POP1 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SOLVENT	0467	0031	6.64	0
		NAP	NF 667 UG/ML	0360	0582	161.67	0
000127479	NA1		0025-2 PCT.	0607	0038	6.26	0
000127479	NA2		0125-3 PCT.	0650	0043	6.62	0
000127479	NA3		0625-4 PCT.	0705	0049	6.95	0

REPORT EXR33 LITTON HIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/25/77			
EXPERIMENT 636503		DETECTOR TA98		SPECIES /			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SOLVENT	1043	0288	27.61	0
		NAP	NF- 667 UG/ML	0890	1371	154.04	0
000127479	NA1		0025-2 PCT.	0482	0020	4.15	0
000127479	NA2		0125-3 PCT.	0490	0033	6.73	0
000127479	NA3		0625-4 PCT.	1339	0062	4.63	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672							
EXPERIMENT 702606	DETECTOR 0000D4	SPECIES	/	DATE - 07/25/77					
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0537	0098	0063	18.25	11.73	0
	NAP		EMS 1.0 %	0365	0587	0456	160.82	124.93	0
000127479	NA1		0017-1 PCT.	0211	0053	0023	25.12	10.90	0
000127479	NA2		0085-2 PCT.	0276	0057	0021	20.65	7.61	0
000127479	NA3		0425-3 PCT.	0438	0082	0034	18.72	7.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705304 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0890	0695	78.09	0
	A-C		SOLVENT	0812	0731	90.02	0
	ALI		TISSUE	0960	0884	92.08	0
	ALU		TISSUE	0685	0587	85.69	0
	ACP	LI	DMN 90 UM/ML	0681	1431	210.13	0
	ACP	LU	DMN 90 UM/ML	0730	0636	87.12	0
000127479	ACT	LI1	0025-2 PCT.	0409	0124	30.32	0
000127479	ACT	LI2	0125-3 PCT.	0604	0600	99.34	0
000127479	ACT	LI3	0625-4 PCT.	0570	0482	84.56	0
000127479	ACT	LU1	0025-2 PCT.	0339	0148	43.66	0
000127479	ACT	LU2	0125-3 PCT.	0393	0244	62.09	0
000127479	ACT	LU3	0625-4 PCT.	0670	0390	58.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 704041 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0656	0025	3.81	0
	A-C		SOLVENT	0430	0025	5.81	0
	ALI		TISSUE	0617	0031	5.02	0
	ALI		TISSUE	0645	0033	5.12	0
	ACP	LI	DMN 90 UM/ML	0490	0796	162.45	0
	ACP	LU	DMN 90 UM/ML	0586	0046	7.85	0
000127479	ACT	LI1	0025-2 PCT.	0253	0011	4.35	0
000127479	ACT	LI2	0125-3 PCT.	0386	0012	3.11	0
000127479	ACT	LI3	0625-4 PCT.	0377	0016	4.24	0
000127479	ACT	LU1	0025-2 PCT.	0287	0011	3.83	0
000127479	ACT	LU2	0125-3 PCT.	0681	0016	2.35	0
000127479	ACT	LU3	0625-4 PCT.	0421	0013	3.09	0

REPORT EXR33 LITTON BIOTNETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701803 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0553	0038	6.87	0
	A-C		SOLVENT	0520	0041	7.88	0
	ALI		TISSUE	0712	0052	7.30	0
	ALU		TISSUE	0518	0037	7.14	0
	ACP	LI	AMQ 333 UG/ML	0329	0312	94.83	0
	ACP	LU	AMQ 333 UG/ML	0622	0036	5.79	0
000127479	ACT	LI1	0025-2 PCT.	0552	0014	2.54	0
000127479	ACT	LI2	0125-3 PCT.	0595	0017	2.86	0
000127479	ACT	LI3	0625-4 PCT.	0671	0023	3.43	0
000127479	ACT	LU1	0025-2 PCT.	0494	0012	2.43	0
000127479	ACT	LU2	0125-3 PCT.	0534	0010	1.87	0
000127479	ACT	LU3	0625-4 PCT.	0795	0040	5.03	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 611108 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0704	0107	15.20	0
	A-C		SOLVENT	0582	0108	18.56	0
	ALI		TISSUE	0475	0059	12.42	0
	ALU		TISSUE	0540	0105	19.44	0
	ACP	LI	ANTH 67 UG/ML	0167	0689	412.57	0
	ACP	LU	ANTH 67 UG/ML	0261	0229	87.74	0
000127479	ACT	LI1	0025-2 PCT.	0511	0065	12.72	0
000127479	ACT	LI2	0125-3 PCT.	0420	0074	17.62	0
000127479	ACT	LI3	0625-4 PCT.	0736	0074	10.05	0
000127479	ACT	LU1	0025-2 PCT.	0542	0072	13.28	0
000127479	ACT	LU2	0125-3 PCT.	0647	0065	10.05	0
000127479	ACT	LU3	0625-4 PCT.	0607	0069	11.37	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 700301 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0609	0059	9.69	0
	A-C		SOLVENT	0533	0075	14.07	0
	ALI		TISSUE	0517	0156	30.17	0
	ALU		TISSUE	0705	0148	20.99	0
	ACP	LI	ANTH 67 UG/ML	1285	1746	135.88	0
	ACP	LU	ANTH 67 UG/ML	0718	0403	56.13	0
000127479	ACT	LI1	0025-2 PCT.	0199	0030	15.08	0
000127479	ACT	LI2	0125-3 PCT.	0248	0034	13.71	0
000127479	ACT	LI3	0625-4 PCT.	0432	0050	11.57	0
000127479	ACT	LU1	0025-2 PCT.	0415	0036	8.67	0
000127479	ACT	LU2	0125-3 PCT.	0937	0025	2.67	0
000127479	ACT	LU3	0625-4 PCT.	0361	0080	22.16	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702605 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1271	0018	0011	1.42	0.87	0
	A-C		SOLVENT	1569	0035	0028	2.23	1.78	0
	ALI		TISSUE	1030	0087	0038	8.45	3.69	0
	ALU		TISSUE	1283	0084	0060	6.55	4.68	0
	ACP	LI	DMN 90 UM/ML	0737	1027	0553	139.35	75.03	0
	ACP	LU	DMN 90 UM/ML	0958	0079	0026	8.25	2.71	0
000127479	ACT	LI1	0017-1 PCT.	1098	0042	0014	3.83	1.28	0
000127479	ACT	LI2	0085-2 PCT.	1357	0063	0012	4.64	0.88	0
000127479	ACT	LI3	0425-3 PCT.	1225	0060	0012	4.90	0.98	0
000127479	ACT	LU1	0017-1 PCT.	2000	0038	0011	1.90	0.55	0
000127479	ACT	LU2	0085-2 PCT.	1753	0061	0018	3.48	1.03	0
000127479	ACT	LU3	0425-3 PCT.	1682	0050	0013	2.97	0.77	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705305 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0548	0520	94.89	0
	ALI		TISSUE	0502	0466	92.83	0
	ALU		TISSUE	0441	0453	102.72	0
	ACP	LI	DMN 90 UM/ML	0480	1364	284.17	0
	ACP	LU	DMN 90 UM/ML	0495	0457	92.32	0
000127479	ACT	LI1	0025-2 PCT.	0478	0333	69.67	0
000127479	ACT	LI2	0125-3 PCT.	0539	0459	85.16	0
000127479	ACT	LI3	0625-4 PCT.	0464	0496	106.90	0
000127479	ACT	LU1	0025-2 PCT.	0504	0312	61.90	0
000127479	ACT	LU2	0125-3 PCT.	0484	0419	86.57	0
000127479	ACT	LU3	0625-4 PCT.	0551	0478	86.75	0
	A-C		SOLVENT	0727	0455	62.59	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702701 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0224	0018	8.04	0
	A-C		SOLVENT	0246	0015	6.10	0
	ALI		TISSUE	0207	0021	10.14	0
	ALU		TISSUE	0202	0026	12.87	0
	ACP	LI	DMN 90 UM/ML	0255	0483	189.41	0
	ACP	LU	DMN 90 UM/ML	0191	0018	9.42	0
000127479	ACT	LI1	0025-2 PCT.	0115	0010	8.70	0
000127479	ACT	LI2	0125-3 PCT.	0119	0010	8.40	0
000127479	ACT	LI3	0625-4 PCT.	0309	0013	4.21	0
000127479	ACT	LU1	0025-2 PCT.	0158	0010	6.33	0
000127479	ACT	LU2	0125-3 PCT.	0124	0019	15.32	0
000127479	ACT	LU3	0625-4 PCT.	0243	0016	6.58	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705303 DETECTOR TA1537 SPECIES SPRDAW/NAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0814	0060	7.37	0
	A-C		SOLVENT	0642	0011	1.71	0
	ALI		TISSUE	0593	0021	3.54	0
	ALU		TISSUE	0566	0027	4.77	0
	ACP	LI	AMQ 333 UG/ML	0302	0190	62.91	0
	ACP	LU	AMQ 333 UG/ML	0538	0015	2.79	0
000127479	ACT	LI1	0025-2 PCT.	0365	0015	4.11	0
000127479	ACT	LI2	0125-3 PCT.	0524	0013	2.48	0
000127479	ACT	LI3	0625-4 PCT.	0602	0029	4.82	0
000127479	ACT	LU1	0025-2 PCT.	0412	0011	2.67	0
000127479	ACT	LU2	0125-3 PCT.	0593	0021	3.54	0
000127479	ACT	LU3	0625-4 PCT.	0681	0048	7.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708201 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0530	0021	3.96	0
	A-C		SOLVENT	0540	0035	6.48	0
	ALI		TISSUE	0589	0040	6.79	0
	ALU		TISSUE	0299	0027	9.03	0
	ACP	LI	ANTH 67 UG/ML	0507	0512	100.99	0
	ACP	LU	ANTH 67 UG/ML	0346	0384	110.98	0
000127479	ACT	LI1	0025-2 PCT.	0257	0017	6.61	0
000127479	ACT	LI2	0125-3 PCT.	0313	0030	9.58	0
000127479	ACT	LI3	0625-4 PCT.	0375	0027	7.20	0
000127479	ACT	LU1	0025-2 PCT.	0370	0032	8.65	0
000127479	ACT	LU2	0125-3 PCT.	0474	0034	7.17	0
000127479	ACT	LU3	0625-4 PCT.	0433	0023	5.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672					
EXPERIMENT 701204	DETECTOR TA98	SPECIES SPRDAW/RAT		DATE - 07/25/77			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0875	0072	8.23	0
	A-C		SOLVENT	1066	0060	5.63	0
	ALI		TISSUE	0663	0269	40.57	0
	ALU		TISSUE	0685	0242	35.33	0
	ACP	LI	ANTH 67 UG/ML	0712	1679	235.81	0
	ACP	LU	ANTH 67 UG/ML	0549	0951	173.22	0
000127479	ACT	L11	0025-2 PCT.	0216	0021	9.72	0
000127479	ACT	L12	0125-3 PCT.	0477	0086	18.03	0
000127479	ACT	L13	0625-4 PCT.	0498	0079	15.86	0
000127479	ACT	LU1	0025-2 PCT.	0152	0015	9.87	0
000127479	ACT	LU2	0125-3 PCT.	0434	0091	20.97	0
000127479	ACT	LU3	0625-4 PCT.	0632	0077	12.18	0

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706372 DETECTOR 000004 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0874	0116	0072	13.27	8.24	0
	A-C		SOLVENT	0723	0164	0071	22.68	9.82	0
	ALI		TISSUE	0623	0173	0096	27.77	15.41	0
	ALU		TISSUE	0752	0185	0088	24.60	11.70	0
	ACP	LI	DMN 90 UM/ML	0688	0582	0315	84.59	45.78	0
	ACP	LU	DMN 90 UM/ML	0599	0096	0066	16.03	11.02	0
000127479	ACT	LI1	0017-1 PCT.	0660	0088	0040	13.33	6.06	0
000127479	ACT	LI2	0085-2 PCT.	0733	0102	0056	13.92	7.64	0
000127479	ACT	LI3	0425-3 PCT.	0609	0094	0063	15.44	10.34	0
000127479	ACT	LU1	0017-1 PCT.	0669	0083	0042	12.41	6.28	0
000127479	ACT	LU2	0085-2 PCT.	0511	0086	0042	16.83	8.22	0
000127479	ACT	LU3	0425-3 PCT.	0600	0088	0040	14.67	6.67	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702009 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0490	0349	71.22	0
	A-C		SOLVENT	0854	0210	24.59	0
	ALI		TISSUE	0886	0716	80.81	0
	ALI		TISSUE	0780	0582	74.62	0
	ACP	LI	DMN 90 UM/ML	1113	1931	173.50	0
	ACP	LU	DMN 90 UM/ML	1016	0748	73.62	0
000127479	ACT	LI1	0025-2 PCT.	0489	0033	6.75	0
000127479	ACT	LI2	0125-3 PCT.	1469	0741	50.44	0
000127479	ACT	LI3	0625-4 PCT.	1158	0879	75.91	0
000127479	ACT	LU1	0025-2 PCT.	0474	0012	2.53	0
000127479	ACT	LU2	0125-3 PCT.	1240	0764	61.61	2
000127479	ACT	LU3	0625-4 PCT.	1074	0739	68.81	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701286 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0286	0043	15.03	0
	A-C		SOLVENT	0229	0021	9.17	0
	ALI		TISSUE	0356	0049	13.76	0
	ALU		TISSUE	0333	0058	17.42	0
	ACP	LI	DMN 90 UM/ML	0539	0487	90.35	0
	ACP	LU	DMN 90 UM/ML	0391	0050	12.79	0
000127479	ACT	LI1	0025-2 PCT.	0363	0030	8.26	0
000127479	ACT	LI2	0125-3 PCT.	0360	0022	6.11	0
000127479	ACT	LI3	0625-4 PCT.	0387	0023	5.94	0
000127479	ACT	LU1	0025-2 PCT.	0448	0043	9.60	0
000127479	ACT	LU2	0125-3 PCT.	0495	0036	7.27	0
000127479	ACT	LU3	0625-4 PCT.	0415	0034	8.19	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705501 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0633	0020	3.16	0
	A-C		SOLVENT	0595	0024	4.03	0
	ALI		TISSUE	0546	0031	5.68	0
	ALU		TISSUE	0646	0028	4.33	0
	ACP	LI	AMQ 333 UG/ML	0539	0311	57.70	0
	ACP	LU	AMQ 333 UG/ML	0645	0018	2.79	0
000127479	ACT	LI1	0025-2 PCT.	0497	0016	3.22	0
000127479	ACT	LI2	0125-3 PCT.	0516	0021	4.07	0
000127479	ACT	LI3	0625-4 PCT.	0642	0016	2.49	0
000127479	ACT	LU1	0025-2 PCT.	0498	0017	3.41	0
000127479	ACT	LU2	0125-3 PCT.	0586	0021	3.58	0
000127479	ACT	LU3	0625-4 PCT.	0570	0023	4.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706702 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1571	0028	1.78	0
	A-C		SOLVENT	1483	0041	2.76	0
	ALI		TISSUE	0839	0042	5.01	0
	ALU		TISSUE	0701	0059	8.42	0
	ACP	LI	ANTH 67 UG/ML	1003	1145	114.16	0
	ACP	LU	ANTH 67 UG/ML	0439	0022	5.01	0
000127479	ACT	LI1	0025-2 PCT.	0310	0031	10.00	0
000127479	ACT	LI2	0125-3 PCT.	0517	0061	11.80	0
000127479	ACT	LI3	0625-4 PCT.	0906	0077	8.50	0
000127479	ACT	LU1	0025-2 PCT.	0325	0027	8.31	0
000127479	ACT	LU2	0125-3 PCT.	0593	0067	11.30	0
000127479	ACT	LU3	0625-4 PCT.	0885	0075	8.47	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701209 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0841	0067	7.97	0
	A-C		SOLVENT	0831	0072	8.66	0
	ALI		TISSUE	0545	0169	31.01	0
	ALU		TISSUE	2078	0348	16.75	0
	ACP	LI	ANTH 67 UG/ML	1293	8694	672.39	0
	ACP	LU	ANTH 67 UG/ML	0553	0048	8.68	0
000127479	ACT	LI1	0025-2 PCT.	0152	0019	12.50	0
000127479	ACT	LI2	0125-3 PCT.	0314	0057	18.15	0
000127479	ACT	LI3	0625-4 PCT.	0555	0100	18.02	0
000127479	ACT	LU1	0025-2 PCT.	0576	0041	7.12	0
000127479	ACT	LU2	0125-3 PCT.	0560	0042	7.50	0
000127479	ACT	LU3	0625-4 PCT.	1458	0088	6.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705603 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0783	0162	0065	20.69	8.30	0
	A-C		SOLVENT	0766	0133	0059	17.36	7.70	0
	ALI		TISSUE	1034	0163	0095	15.76	9.19	0
	ALU		TISSUE	1130	0233	0101	20.62	8.94	0
	ACP	LI	DMN 90 UM/ML	0669	0591	0418	88.34	62.48	0
	ACP	LU	DMN 90 UM/ML	0643	0104	0077	16.17	11.98	0
000127479	ACT	LI1	0017-1 PCT.	0901	0126	0059	13.98	6.55	0
000127479	ACT	LI2	0085-2 PCT.	0917	0226	0064	24.65	6.98	0
000127479	ACT	LI3	0425-3 PCT.	0991	0170	0092	17.15	9.28	0
000127479	ACT	LU1	0017-1 PCT.	0930	0131	0059	14.09	6.34	0
000127479	ACT	LU2	0085-2 PCT.	0989	0153	0081	15.47	8.19	0
000127479	ACT	LU3	0425-3 PCT.	0976	0139	0063	14.24	6.45	0